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$p21^{ras}$ activation via hemopoietin receptors and c-kit requires tyrosine kinase activity but not tyrosine phosphorylation of $p21^{ras}$ GTPase-activating protein

(cytokines/growth factor/signal transduction/mast cell)

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ABSTRACT **Products of the *ras* gene family, termed $p21^{ras}$, are GTP-binding proteins that have been implicated in signal transduction via receptors encoding tyrosine kinase domains. Recent findings have defined a superfamily of hemopoietin receptors that includes receptors for a number of interleukins and colony-stimulating factors. The intracellular portions of these receptors show only restricted homologies, have no tyrosine kinase domain, and provide no clues to the mode of signal transduction. However, in most cases the factors stimulate tyrosine phosphorylation. We demonstrate here that ligand-induced activation of the interleukin (IL)-2, IL-3, IL-5, and granulocyte–macrophage colony-stimulating factor receptors resulted in activation of $p21^{ras}$ in various hemopoietic cell lines. The only cytokine tested that binds to a hemopoietin receptor and that did not activate $p21^{ras}$ was IL-4. Activation of $p21^{ras}$ was also observed in response to Steel factor, which stimulates the endogenous tyrosine kinase activity of the c-kit receptor, as well as with phorbol esters, which activate protein kinase C. Experiments with protein kinase inhibitors implicated tyrosine kinase activity, but not protein kinase C activity, as the upstream signal in $p21^{ras}$ activation via these growth factor receptors. Attempts to demonstrate tyrosine phosphorylation of the $p21^{ras}$ GTPase-activating protein (GAP) were negative, suggesting that phosphorylation of GAP may not be the major mechanism for regulation of $p21^{ras}$ activity by tyrosine kinases.**

Hemopoietic cells grow in response to a number of growth factors that are referred to as interleukins (IL) and colony stimulating factors (CSF), all of which share only limited structural similarity and sequence homologies (1). The restricted cell targets of these factors have now been shown to result from their interaction with a superfamily of receptors known as the hemopoietin receptors (for review, see ref. 2). This receptor family shares a number of highly conserved structural features, including a WSXWS motif, four conserved cysteines, and fibronectin binding domains. Binding of many of the factors to their receptors has been shown to activate tyrosine phosphorylation of a number of substrates but, with the possible exception of IL-2 (3), the tyrosine kinase that is activated has not been identified.

A large proportion of human tumors have been shown to express activated versions of the *ras* family of genes, whose products are collectively referred to as $p21^{ras}$ (4). $p21^{ras}$ are GTP-binding proteins that have been shown, by both indirect and direct approaches, to be involved in events downstream of tyrosine kinases. The anti-$p21^{ras}$ monoclonal antibody Y13-259, when microinjected into cells, has been shown to inhibit serum and growth factor-induced stimulation of mitogenesis, as well as transformation by tyrosine kinase-encoding oncogenes (5, 6). Several recent studies (7–9) have shown that both nonreceptor and receptor tyrosine kinases are able to stimulate $p21^{ras}$ activity, as measured by an increase in the amount of $p21^{ras}$ having bound GTP compared to GDP. A similar increase in the ratio of GTP/GDP was observed in T cells after stimulation of the T-cell antigen receptor or in response to phorbol esters (10).

We demonstrate here that IL-2, IL-3, IL-5, granulocyte–macrophage CSF (GM-CSF) and Steel factor (SLF), but not IL-4, activate $p21^{ras}$ in hemopoietic cells. Phorbol esters had a small effect on $p21^{ras}$ activity, but it was much less than that of cytokines acting on the same cells. Furthermore, experiments with inhibitors of protein tyrosine kinases and of serine/threonine kinases suggest that tyrosine kinase activity, but not protein kinase C (PKC) activity, is required for this stimulation. Initial attempts to characterize the potential site of action of the tyrosine kinases focused on GTPase-activating protein (GAP) (11, 12), but no increase in its phosphorylation was observed with any of the factors tested. Therefore, the primary effect of tyrosine kinase activity in stimulation of $p21^{ras}$ activity by the hemopoietic growth factors may be via tyrosine phosphorylation of other proteins involved in the regulation of $p21^{ras}$ activity.

MATERIALS AND METHODS

Materials. The following materials were from the sources indicated: RPMI 1640 and other media (Terry Fox Media Preparation Service, Vancouver, BC); [^{32}P]orthophosphate (ICN); alkaline phosphatase-conjugated goat anti-mouse IgG (Calbiochem); 5-bromo-4-chloro-3-indolylphosphate (BCIP) and nitroblue tetrazolium chloride (NBT) (GIBCO/BRL); nitrocellulose (Schleicher & Schuell); protein A-Sepharose 4B (Pharmacia); 4G10 anti-phosphotyrosine antibody (Upstate Biotechnology, Lake Placid, NY). Synthetic forms of murine IL-3, IL-4, GM-CSF, and SLF were synthesized with an Applied Biosystems instrument as described (13). Recombinant IL-2 and IL-5 were produced in a B-cell hybridoma cell line, X63Ag8-653 (from F. Melchers, Basel, Switzerland), transfected with a bovine papilloma virus-based expression vector (14). Each of the factors used was shown to be free of contaminating activity. The Y13-259 hybridoma (15) was a kind gift from N. Auersperg (Univ. of British Columbia). Anti-GAP antiserum was a kind gift from C. Ellis and T. Pawson (Mt. Sinai Research Institute, Toronto). Tyrosine kinase inhibitors were a generous gift from Alan Hudson (Wellcome).

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Abbreviations: GAP, $p21^{ras}$ GTPase-activating protein; IL, interleukin(s); GM-CSF, granulocyte–macrophage colony stimulating factor; PDBU, phorbol 12,13-dibutyrate; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; SLF, Steel factor; PDGF, platelet-derived growth factor.

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Cell Culture. R6X is a mast/megakaryocyte cell line isolated from mouse bone marrow cultures grown in the presence of IL-3 (16). A clone of FDC-P1 (17) that responds to both IL-3 and GM-CSF was obtained from A. J. Hapel (Canberra, Australia). MC-9 is a mast cell line that responds to IL-3, IL-4, IL-5, GM-CSF, and SLF. These three lines were routinely passaged in RPMI 1640 medium containing 10% fetal bovine serum, 20 μM 2-mercaptoethanol, and 2% 10-fold concentrated medium conditioned by WEHI-3 cells. FDC-2 cells were obtained from J. D. Watson (18) and were grown in the presence of IL-2. For all experiments, cells were starved overnight by replacing 90% of the volume with medium containing no added growth factors.

Assay of p21^{ras} Activity. Cells were washed three times in phosphate-free RPMI 1640 medium and incubated at 37°C in the same medium buffered with 20 mM Hepes (pH 7.4) using 1.0×10^7 cells per ml and 0.3–0.5 mCi of [^{32}P]orthophosphate per ml (1 Ci = 37 GBq). After 2 hr of labeling, cells were treated as indicated, washed once with ice-cold phosphate-buffered saline (PBS), and immunoprecipitated using Y13-259 as described (10). Briefly, protein A-Sepharose beads were incubated in PBS containing 1.0 mg of bovine serum albumin per ml and a 1:50 dilution of rabbit anti-rat IgG antiserum followed by incubation with 10 μg of Y13-259 antibody per ml; samples were immunoprecipitated with 15 μl of antibody-coated packed beads. Bound GTP and GDP were eluted as described (10) and separated on polyethyleneimine-cellulose TLC sheets developed in 1.0 M KH_2PO_4 (pH 3.4) (19). After autoradiography, the ratio of GTP/GDP was determined by a laser scanning densitometer (Molecular Dynamics). The response of the densitometer was shown to be linear over the range of radioactivity present in the GDP and GTP spots (10–250 cpm).

GAP Phosphorylation. GAP was immunoprecipitated from extracts of cells treated as indicated, essentially as described (20). The GAP immunoprecipitates were analyzed by immunoblotting using 4G10 anti-phosphotyrosine antibody. Nitrocellulose blots were incubated overnight in blocking buffer containing 20 mM Tris·HCl (pH 7.5), 0.15 M NaCl, and 5% bovine serum albumin, 1% ovalbumin, and 0.05% sodium azide. Incubations with anti-phosphotyrosine antibody were in buffer containing 1% bovine serum albumin plus 0.5 μg of 4G10 per ml for 2 hr. Blots were washed and incubated in alkaline phosphatase-conjugated secondary antibody (1:2000 dilution of stock) for 1 hr. Color development was in 10 mM Tris·HCl, pH 9.5/100 mM NaCl/5 mM $MgCl_2$ plus BCIP and NBT, as suggested by the manufacturer. As a positive control for tyrosine phosphorylation of GAP, Rat-1 cells transformed with the PrA strain of wild-type v-*src* (21) were tested in the same assay.

RESULTS

IL-3 Rapidly Stimulates Increased GTP Association with p21^{ras}. The IL-3-dependent cell line R6X was treated for various times with a saturating concentration of synthetic IL-3. As indicated in Fig. 1, there was an increase in the amount of GTP associated with p21^{ras} within 1 min and this reached a maximum level by 10 min. After 20 min of IL-3 treatment, the ratio of GTP/GDP had decreased. This result was seen consistently in several experiments, with the maximal effect observed between 5 and 10 min of treatment. When R6X cells were analyzed for IL-3-induced tyrosine phosphorylation, very similar kinetics of phosphorylation were observed, reaching a maximal level by 10 min and decreasing by 20 min (V.D., I. Clark-Lewis, B. Federsppiel, J. S. Wieler, and J.W.S., unpublished observations).

Cytokine-Induced Stimulation of p21^{ras} Activity in a Number of Hemopoietic Cell Lines. The mast cell line MC-9 was useful for testing a number of cytokines, since the cells grow in either IL-3, IL-5, GM-CSF, or SLF, and they respond in short-term proliferation assays to IL-4. Each of the factors, with the exception of IL-4, was able to stimulate p21^{ras} (Fig. 2). Table 1 summarizes these and other results using various cells and treatments. FDC-2 cells were found to respond to IL-2 as well as to IL-3. Similar to the results in MC-9, IL-4 did not have an effect on p21^{ras} in either FDC-2 or R6X. In all three cases, the cells are kept alive, but with only modest proliferation in the presence of IL-4. Phorbol esters were also tested in R6X and FDC-P1 cells and were found to increase the GTP/GDP ratio, although their effect was consistently less than that of the cytokines. Phorbol esters were also unable to stimulate growth of these cells.

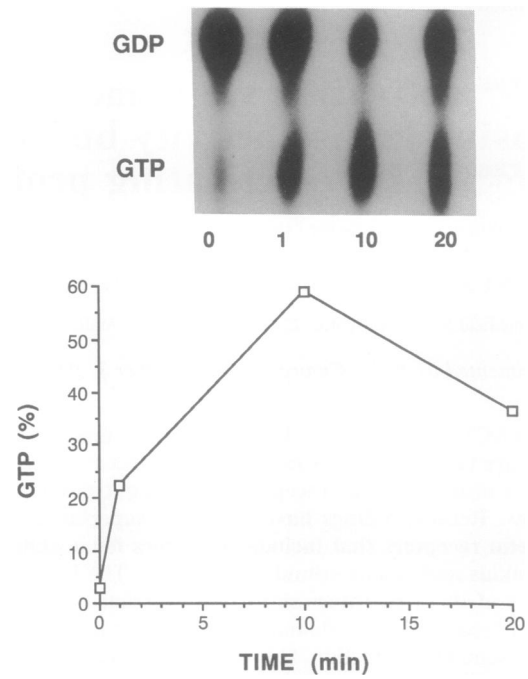

FIG. 1. Time course of p21^{ras} activation by IL-3. R6X cells were treated for the indicated times (min) with IL-3 and guanine nucleotides bound to p21^{ras} were analyzed as described. An autoradiogram of the TLC plate from a representative experiment is shown, along with the corresponding ratio of GTP/GDP as determined by densitometry.

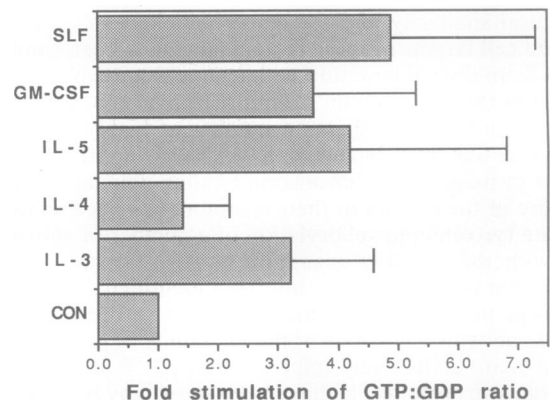

FIG. 2. Effect of various cytokines on MC-9 cells. Cells grown in IL-3 were incubated overnight in reduced factor and, after ^{32}P labeling, cells were either untreated (CON) or treated with IL-3, IL-4, IL-5, or GM-CSF for 10 min, or with SLF for 2 min. In each case, the time and concentration of factors were based on the optimal tyrosine phosphorylation response. Values represent the average of four independent experiments ± SD. The control value in each experiment was normalized to 1.0; the average value for the four control samples was 5.8% ± 3.2% GTP.

Table 1. $p21^{ras}$ activity in hemopoietic cells

Cell type	Treatment	Increase in % GTP
R6X	IL-3	++++
	IL-4	−
	PDBU	+
FDC-P1	IL-3	++++
	GM-CSF	+++
	PMA	++
FDC-2	IL-3	++
	IL-4	−
	IL-2	++
MC-9	IL-3	++
	IL-4	−
	IL-5	+++
	SLF	+++
	GM-CSF	++

Chemically synthesized IL-3, IL-4, GM-CSF, and SLF were used; sources of IL-2 and IL-5 were supernatants of transfected cells. In each case, parallel experiments demonstrated that the same preparations of cytokines stimulated tyrosine phosphorylation of a characteristic pattern of substrates. Cytokines were added at concentrations and times known to stimulate maximum tyrosine phosphorylation. For SLF, the time was 2 min and for all others it was 10 min. PMA (10 nM) was added for 20 min and PDBU (100 nM) was added for 20 min. Results are presented in terms of the increase in $p21^{ras}$-associated GTP as a percentage of total bound guanine nucleotides (0%, no GTP bound; 100%, no GDP bound). −, Treatments that resulted in an increase of <5%; +, increase of 5–10%; ++, increase of 10–20%; +++, increase of 20–30%; ++++, increase of >30%. In most experiments, control cells had <5% GTP (and often <1%) associated with the immunoprecipitated $p21^{ras}$. Each experiment was repeated at least twice, and in most cases at least three times, with similar results.

Inhibition of Tyrosine Kinase, but not PKC Activity, Blocked $p21^{ras}$ Activation. We used a series of tyrosine kinase and PKC inhibitors to evaluate their effects in inhibiting $p21^{ras}$ activation. The first result suggesting the involvement of tyrosine kinase activity was obtained by using staurosporine (22), an inhibitor of numerous protein kinases, and a structurally related inhibitor, no. 3, originally reported by Davis *et al.* (23) to be a more specific inhibitor of PKC. As shown in Fig. 3, staurosporine was a potent inhibitor of IL-3 induced $p21^{ras}$ activation, while the PKC inhibitor no. 3 had almost no effect. The effectiveness of the inhibitors on PKC was confirmed by their inhibition of PKC activity in extracts of cells pretreated with inhibitors, as well as by their ability to inhibit phorbol ester-induced $p21^{ras}$ activation (results not shown). Therefore, the IL-3-induced activation of $p21^{ras}$ did not appear to be dependent on PKC activity. We have also observed that staurosporine, at concentrations equal to or lower than those used here, was a very potent inhibitor of tyrosine kinase activity stimulated by IL-3, whereas the PKC inhibitor no. 3 had no effect on tyrosine phosphorylation (results not shown). The latter observations led us to test a number of other tyrosine kinase inhibitors.

Two tyrosine kinase inhibitors, ST271 and ST638 (24), inhibited IL-3-induced increases in tyrosine phosphorylation while two other inhibitors, coded as nos. 25 and 47 by Gazit *et al.* (25), were found to be much less effective (results not shown). As shown in Fig. 4, ST271 and ST638 were very effective at blocking IL-3-stimulated $p21^{ras}$ activation. The two inhibitors, nos. 25 and 47, were unable to block IL-3-stimulated $p21^{ras}$ activation, correlating with their inability to block IL-3-induced tyrosine phosphorylation (results not shown). Similar experiments showed that ST271 and ST638 also blocked SLF-induced tyrosine phosphorylation and another tyrosine kinase inhibitor, genistein, was an even more potent inhibitor (data not shown). As shown in Fig. 4, genistein was a potent inhibitor of SLF-stimulated $p21^{ras}$ activation. ST271 and ST638 were also found to inhibit the SLF-induced effect when tested in one experiment. The tyrosine kinase inhibitors had no effect on the basal level of GTP bound to $p21^{ras}$ when they were added alone to the cells (data not shown).

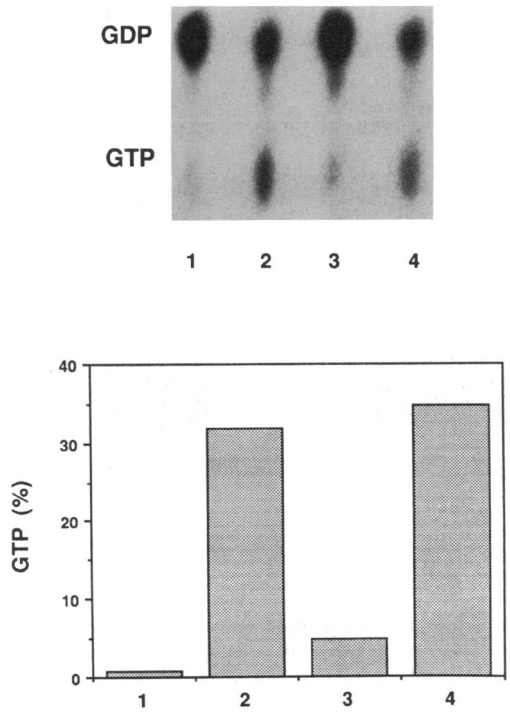

FIG. 3. Effect of kinase inhibitors on $p21^{ras}$ activation. FDC-P1 cells grown in IL-3 were incubated overnight in reduced factor, ^{32}P labeled, and $p21^{ras}$ immunoprecipitated as described. Cell treatments were as follows: lane 1, untreated; lane 2, treated with IL-3 for 10 min; lane 3, pretreated for 2 min with 1 μM staurosporine followed by IL-3 for 10 min; lane 4, pretreated with 10 μM PKC inhibitor no. 3 (23) for 2 min followed by IL-3 for 10 min. These results are from a representative experiment that was repeated three times. Similar results were also obtained when cells were preincubated with the inhibitors for up to 30 min.

Tyrosine Phosphorylation of GAP Is Not Detected in Response to the Various Cytokines. Previous studies have suggested a role for tyrosine phosphorylation of GAP in the regulation of $p21^{ras}$. We tested this in our system by immunoprecipitation of GAP from cytokine-treated cells (Fig. 5).

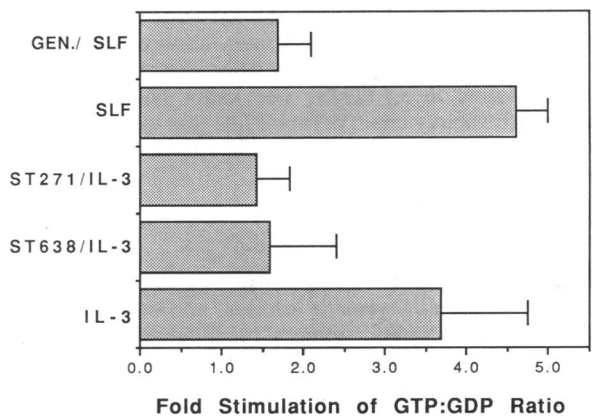

FIG. 4. Inhibition of $p21^{ras}$ activation by tyrosine kinase inhibitors. Cells were treated with IL-3 for 10 min or SLF for 2 min, or they were pretreated with tyrosine kinase inhibitors ST271, ST638, or genistein (GEN.) for 30 min followed by IL-3 or SLF as indicated. Control values were normalized to 1.0.

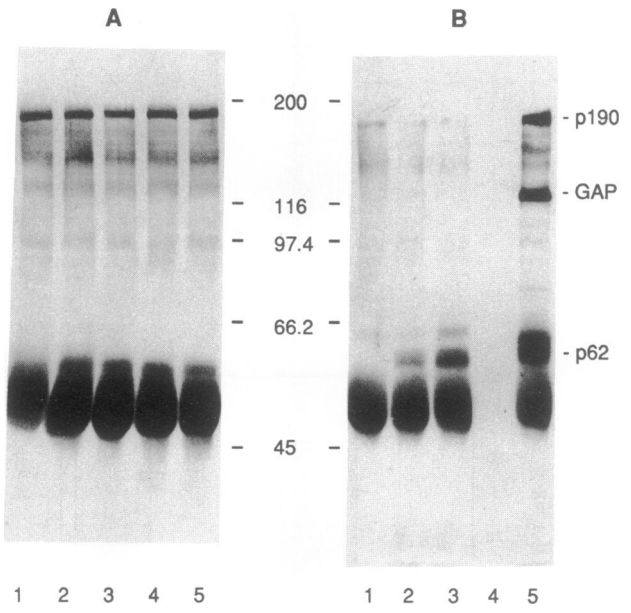

FIG. 5. Immunoprecipitation and anti-phosphotyrosine blot of GAP. (*A*) MC-9 cells (1.0×10^7 cells) were either untreated (lane 1) or treated with SLF for 2 min (lane 2), GM-CSF for 10 min (lane 3), IL-4 for 10 min (lane 4), or IL-5 for 10 min (lane 5). GAP immunoprecipitates were separated on a 7.5% polyacrylamide gel, transferred to nitrocellulose, and blotted using anti-phosphotyrosine antibody 4G10. (*B*) R6X cells were either untreated (lane 1) or treated with IL-3 for 10 min (lanes 2 and 3), and cell extracts were immunoprecipitated with anti-GAP antibodies. Extracts used for immunoprecipitation were from 1.0×10^7 cells (lanes 1 and 2) and from 4.0×10^7 cells (lane 3). Lane 4, blank; lane 5, GAP immunoprecipitate from $\approx 1.0 \times 10^6$ Rat-1 cells transformed with v-src.

MC-9 cells were treated with either IL-4, IL-5, GM-CSF, or SLF (Fig. 5*A*), but in no case was there any detectable phosphorylation of GAP. Fig. 5*B* shows that stimulation of R6X cells with IL-3, followed by immunoprecipitation of GAP, also did not reveal any tyrosine-phosphorylated bands at the position expected for GAP. The same was true even when 4 times as many cells were used. As a positive control, it was shown that tyrosine-phosphorylated GAP, as well as p190 and p62, could be detected in an immunoprecipitate from only 1.0×10^6 v-*src*-transformed Rat-1 cells, as has been reported (26). In addition, we demonstrated by blotting with anti-GAP antibodies that GAP had been immunoprecipitated in all cases (results not shown).

In no case was there any increase in the phosphorylation of p190 after stimulation with the cytokines. On the other hand, there was increased phosphorylation of a coimmunoprecipitated polypeptide at the position expected for p62 and this was found in all cases. The major bands below p62 represent the heavy chain of rabbit IgG that was detected due to the cross-reactivity of alkaline phosphatase-coupled goat anti-mouse IgG.

DISCUSSION

The activation of $p21^{ras}$ in response to a number of growth factors, nonreceptor tyrosine kinases, and other cell stimuli has been demonstrated in several recent studies by assaying the increase in bound GTP associated with immunoprecipitated $p21^{ras}$ (7–10, 27, 28). The results presented here show that a number of cytokines—IL-2, IL-3, IL-5, and GM-CSF—that bind to hemopoietin receptors were able to activate $p21^{ras}$. Another cytokine, SLF (29), which acts on similar cell types but is the ligand for a tyrosine kinase-encoding receptor, c-kit, had a similar effect. Since platelet-derived growth factor (PDGF) has also been shown to activate $p21^{ras}$ (7, 8), the latter result was not surprising, as the c-kit receptor protein is related to the PDGF receptor (30). It was intriguing that only one of the cytokines tested, IL-4, gave a negative result. During the course of our study, Satoh *et al.* (28) reported that some of the same cytokines we had used—IL-2, IL-3, and GM-CSF—were able to activate $p21^{ras}$, but IL-4 was not, even in a cell line growing in IL-4. Their study provided no characterization of the potential mechanisms involved in the activation. In our study, the IL-4-responsive cells showed only a limited proliferative response to the factor. Our attempts to obtain IL-4-dependent cells were unsuccessful, resulting only in factor-independent cells, so we have not been able to verify that IL-4 is unable to activate $p21^{ras}$ in an IL-4-dependent cell line.

One of the key aspects of $p21^{ras}$ activation that remains unresolved is the mechanism by which protein kinases are involved in its regulation. With the recent suggestion that PKC activity may be important in this process (10), we attempted to dissect the phosphorylation events that are necessary for $p21^{ras}$ activation in hemopoietic cells. The kinetics of $p21^{ras}$ activation correlated well with the stimulation of tyrosine phosphorylation by both IL-3 (V.D., I. Clark-Lewis, B. Federsppiel, J. S. Wieler, and J.W.S., unpublished observations) and SLF (M.J.W. and J.W.S., unpublished observations). We tested the effects of various inhibitors of protein kinases on IL-3-stimulated activation of $p21^{ras}$. The inhibition of tyrosine phosphorylation by several tyrosine kinase inhibitors correlated with their ability to inhibit $p21^{ras}$ activation while a PKC inhibitor had no effect. We observed that phorbol 12-myristate 13-acetate (PMA) and phorbol 12,13-dibutyrate (PDBU) did activate $p21^{ras}$ in the factor-dependent cell lines R6X and FDC-P1, similar to the results of Downward *et al.* (10) with T cells. However, the degree of activation was much smaller and the time required for activation was much longer than that found in response to cytokines. These results suggest that activation of PKC is not involved in the initial activation of $p21^{ras}$ after stimulation via hemopoietin receptors, but activation of tyrosine phosphorylation is necessary.

Since both receptor and nonreceptor tyrosine kinases are able to stimulate the tyrosine phosphorylation of GAP (27, 28, 31, 32), one might have assumed that GAP was a primary target of tyrosine kinases that influence $p21^{ras}$ activity. However, GAP that is associated with p190 has been shown to have reduced activity, but this GAP is primarily serine phosphorylated (32). If tyrosine phosphorylation of GAP or GAP-associated proteins were able to inhibit its GTPase-activating activity, then the rate of GTP conversion to GDP would be decreased, and GDP–GTP exchange would result in increased $p21^{ras}$ activity. As has been demonstrated in our study, although all of the factors we have tested are able to stimulate tyrosine phosphorylation of numerous substrates, the phosphorylation of GAP could not be detected. Likewise, Rottapel *et al.* (33) were unable to see GAP phosphorylation in response to SLF. Therefore, if tyrosine phosphorylation is directly affecting $p21^{ras}$ activity, as suggested by the rapid time course of activation and the effect of tyrosine kinase inhibitors, then there must be tyrosine-phosphorylated proteins other than GAP that are involved. Alternatively, GAP could be regulated by serine or threonine phosphorylation, as has been reported (10), but such an effect would not have been detected in our assays. It is interesting that of the two polypeptides, p190 and p62, that associate with GAP (26, 32), p190 was tyrosine phosphorylated to the same extent in all samples, but there was increased tyrosine phosphorylation of p62 in factor-treated compared to control cells. The other interesting aspect of these results is that IL-4 was as effective as the other factors in stimulating tyrosine phosphorylation of p62, so its increased tyrosine phosphorylation is not sufficient for $p21^{ras}$ activation. Other potential targets of tyrosine

phosphorylation include the recently described nucleotide exchange-promoting protein (34, 35) and the neurofibromatosis gene product (36, 37). Whereas the latter has activity similar to GAP, which can only cause inactivation of $p21^{ras}$, a nucleotide exchange protein may provide a target for positive regulation of $p21^{ras}$ activity.

While activation of $p21^{ras}$ is common to a number of signal transduction pathways, its role in cell regulation is still poorly understood. The negative results with IL-4 in an IL-4-grown cell line (28) cause it to stand out as the only cytokine that does not stimulate $p21^{ras}$ activity in hemopoietic cells. IL-4 stimulates tyrosine phosphorylation (refs. 38 and 39; V.D., unpublished observations), but very few cell types grow in response to IL-4, so it will be important to determine how those that do might have bypassed the need for $p21^{ras}$ activation. The activation of $p21^{ras}$ by phorbol esters and via the T-cell receptor (10) also demonstrate that this effect is not seen exclusively as a consequence of, or in conjunction with, growth of cells. In the case of $p21^{ras}$ activation by PKC, there is a possibility that this activation is not a primary event in response to growth factors, but it is a secondary phenomenon that may be involved in prolonging an initial activation event. While in many cases growth factors that interact with tyrosine kinase-encoding receptors can activate $p21^{ras}$, one exception is the study by Burgering *et al.* (40) with insulin, PDGF, and epidermal growth factor, which yielded results opposite those of Satoh *et al.* (8, 9) using similar cell types.

The activation of $p21^{ras}$ appears to be a point of convergence for signaling via IL-2, -3, and -5, and GM-CSF and SLF. In another study, we have demonstrated that IL-3, IL-5, GM-CSF and SLF, but not IL-4, promote tyrosine phosphorylation and activation of members of the microtubule-associated protein (MAP) kinase family of serine/threonine protein kinases (M.J.W., V.D., J. S. Sanghera, S. L. Pelech, and J.W.S., unpublished observations). Future work needs to concentrate on the relationship between these two distinct points in the signal transduction pathways downstream of hemopoietic growth factor receptors, especially the tyrosine phosphorylation events that may be critical for each.

In summary, stimulation of $p21^{ras}$ is observed in response to various hemopoietic growth factors that act via either hemopoietin receptors or tyrosine kinase-encoding receptors in several hemopoietic cell types. The activation of $p21^{ras}$ appears to require tyrosine phosphorylation, but not PKC activity, based on studies with protein kinase inhibitors. In our system, we cannot detect tyrosine phosphorylation of GAP, suggesting that other targets of tyrosine phosphorylation are involved in the activation of $p21^{ras}$.

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